

## AMENDMENTS ( Art. 34 )

(Amendments according to the rules provided in regulation clause 11)

### 1. International Application Number

PCT/JP2004/004190

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### 4. Area of Amendment

Description and Claims

### 5. Amendment

(1) Description p.2 ll.32 to 34: "a polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3" to be amended to "a polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2, and forming an intermolecular disulfide bond between two of the cysteines contained in SEQ ID NO:1 or SEQ ID NO:2".

(2) Description, p.3, ll.2 to 4: "a polypeptide or salts thereof comprising an amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3" to be amended to "a polypeptide or salts thereof comprising an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2, but not consisting of an amino acid sequence represented by SEQ ID NO:4, and forming an intermolecular disulfide bond between two of the cysteines contained in SEQ ID NO:1 or SEQ ID NO:2".

(3) Description, p.3, ll.5 to 9: "a polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:16 or SEQ ID NO:17. These polypeptides, as confirmed in the embodiment of this description, are polypeptides that show mechano-sensitive channel inhibiting activity. These polypeptides are useful for the treatment of atrial fibrillation and such" to be amended to "a polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2, of which one or several of the amino acids thereof have been deleted, substituted, inserted or added, also forming an intermolecular disulfide bond and moreover showing mechano-sensitive channel inhibiting activity".

(4) Description, p.3, ll.10 to 14: "a polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3 of which one or more of the amino acids thereof have been deleted, substituted, inserted or added, but not consisting of an amino acid sequence described in SEQ ID NO:4 and moreover showing mechano-sensitive channel inhibiting activity" to be amended to "the

polypeptide or salts thereof described in the above [3] as consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2 of which one or more of the amino acids thereof have been deleted, substituted, inserted or added, of which the said amino acid sequence is an amino acid sequence represented by SEQ ID NO:16 or SEQ ID NO:17”.

(5) Description, p.3, ll.15 to 21: “a polypeptide or salt thereof described in the above [4] as consisting of an amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3 of which one or more of the amino acids thereof have been deleted, substituted, inserted or added, not consisting of an amino acid sequence described in SEQ ID NO:4 and moreover showing mechano-sensitive channel inhibiting activity, of which the said polypeptide comprises an amino acid sequence represented by SEQ ID NO:16 or SEQ ID NO:17” to be amended to “a polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:16 or SEQ ID NO:17. These polypeptides, as confirmed in the embodiment of this description, are polypeptides that show mechano-sensitive activity. These polypeptides are useful for treatment of atrial fibrillation and such.”

(6)

(7) Description, p.3, ll.22 to 24: “a polynucleotide comprising the polynucleotide that encodes the polypeptide described in the above [1], the above [3] or the above [4]” to be amended to “a polynucleotide comprising the polynucleotide that encodes the polypeptide described in the above [1] as consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2, and forming an intermolecular disulfide bond between two of the cysteines contained in SEQ ID NO:1 or SEQ ID NO:2, of which the said polypeptide comprises an amino acid sequence represented by SEQ ID NO:2, encodes the polypeptide described in the above [3], or encodes the polypeptide described in the above [5]”.

(8) Claim 1 “A polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3,” to be amended to “A polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2, and forming an intermolecular disulfide bond between two of the cysteines contained in SEQ ID NO:1 or SEQ ID NO:2”.

(9) Claim 2 “A polypeptide or salts thereof comprising an amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3” to be amended to “A polypeptide or salts thereof comprising an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2, but not consisting of an amino acid sequence represented by SEQ ID NO:4, and forming an intermolecular disulfide bond between two of the cysteines contained in the amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2”.

(10) Claim 3 “A polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:16 or SEQ ID NO:17” to be amended to “A polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2 of which one or several of the amino acids thereof have been deleted, substituted, inserted or added, also forming an intermolecular disulfide bond and moreover showing mechano-sensitive channel inhibiting activity”.

(11) Claim 4 “A polypeptide or salts thereof consisting of an amino acid

sequence represented by SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3 of which one or more of the amino acids have been deleted, substituted, inserted or added, but not consisting of an amino acid sequence described in SEQ ID NO:4 and moreover showing mechano-sensitive channel inhibiting activity,” to be amended to “A polypeptide or salts thereof described in the above claim 3 as consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2 of which one or several of the amino acids thereof have been deleted, substituted, inserted or added, of which the said amino acid sequence is an amino acid sequence represented by SEQ ID NO:16 or SEQ ID NO:17”.

(12) Claim 5 “A polypeptide or salts thereof described in the above claim 4 as consisting of an amino acid sequence represented by SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3 of which one or more of the amino acids thereof have been deleted, substituted, inserted or added, but not consisting of an amino acid sequence described in SEQ ID NO:4 and moreover showing mechano-sensitive channel inhibiting activity, of which the said polypeptide comprises an amino acid sequence represented by SEQ ID NO:16 or SEQ ID NO:17” to be amended to “A polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:16 or SEQ ID NO:17”.

(13) Claim 6 “A polynucleotide comprising the polynucleotide that encodes the polypeptide described in claim 1, claim 3 or claim 4” to be amended to “A polynucleotide comprising the polynucleotide that encodes the polypeptide described in claim 1 as consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2 and forming an intermolecular disulfide bond between two of the cysteines contained in SEQ ID NO:1 or SEQ ID NO:2, of which the said polypeptide comprises an amino acid sequence represented by SEQ ID NO:2, encodes the polypeptide described in claim 3, or encodes the polypeptide described in claim 5”.

6. List of the accompanying documents.

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| (1) Document replacing Description p.3 and 4 | 1 copy |
| (2) Document replacing Claims p.21           | 1 copy |

The object of the present invention is to identify the pharmacophore (the minimum space structure needed for activation) of GsMTx-4, to design novel polypeptides that specifically inhibit the activity of a mechano-sensitive channel based on the pharmacophore information, and to provide  
5 remedies for atrial fibrillation consisting of such polypeptides.

## SUMMARY OF INVENTION

The above objects are achieved by the following inventions.

[1] In a first aspect of the present invention, it involves a polypeptide or salts  
10 thereof consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2, and forming an intermolecular disulfide bond between two of the cysteines contained in SEQ ID NO:1 or SEQ ID NO:2. These polypeptides, as confirmed in the embodiment of this description, are polypeptides that show mechano-sensitive channel inhibiting activity, and can  
15 be considered as polypeptides that compose the pharmacophore of GsMTx-4. These polypeptides are useful for treatment of atrial fibrillation and such.

[2] In a second aspect of the present invention, it involves a polypeptide or salts thereof comprising an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2, not having an amino acid sequence represented by SEQ ID  
20 NO:4, and forming an intermolecular disulfide bond between two of the cysteines contained in SEQ ID NO:1 or SEQ ID NO:2.

[3] In a third aspect of the present invention, it involves a polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2, of which one or several of the amino acids thereof have been  
25 deleted, substituted, inserted or added, also forming an intermolecular disulfide bond and moreover showing mechano-sensitive channel inhibiting activity.

[4] In a fourth aspect of the present invention, it involves the polypeptide or salts thereof described in the above [3] as consisting of an amino acid

30 sequence represented by SEQ ID NO:1 or SEQ ID NO:2 of which one or more

of the amino acids thereof have been deleted, substituted, inserted or added, of which the said amino acid sequence is an amino acid sequence represented by SEQ ID NO:16 or SEQ ID NO:17.

[5] In a fifth aspect of the present invention, it involves a polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:16 or SEQ ID NO:17. These polypeptides, as confirmed in the embodiment of this description, are polypeptides that show mechano-sensitive activity. These polypeptides are useful for treatment of atrial fibrillation and such.

[6] In a sixth aspect of the present invention, it involves a polynucleotide comprising the polynucleotide that encodes the polypeptide described in the above [1] as consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2, and forming an intermolecular disulfide bond between two of the cysteines contained in SEQ ID NO:1 or SEQ ID NO:2, of which the said polypeptide comprises an amino acid sequence represented by SEQ ID NO:2, encodes the polypeptide described in the above [3], or encodes the polypeptide described in the above [5].

[7] In a seventh aspect of the present invention, it involves a recombinant vector comprising the polynucleotide described in the above [6].

[8] In a eighth aspect of the present invention, it involves a transformant transformed by the recombinant vector described in the above [7].

[9] In a ninth aspect of the present invention, it involves a mechano-sensitive channel inhibitor comprising one or more of the polypeptides or salts thereof described in one of the above [1] to [5]. This inhibitor specifically inhibits the activity of a mechano-sensitive channel and thus is useful for conducting researches on mechano-sensitive channels and such.

[10] In a tenth aspect of the present invention, it involves a remedy for atrial fibrillation comprising one or more of the polypeptides or salts thereof described in one of the above [1] to [5]. These polypeptides, as confirmed of their functions in the embodiment of this description, show mechano-sensitive channel inhibiting activity. Therefore, this remedy can be used

effectively in treating atrial fibrillation.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a representation of the ten prospective multiple alignments  
5 showing high homology to GsMTx-4

Fig. 2 is a representation of the alignment of IQK6 and GsMTx-4.

Fig. 3 is a stereo view superimposing Huwentoxin-I and GsMTx-4.

Fig. 4 is a Ca trace of the model of the superimposed Huwentoxin-I  
and GsMTx-4. In Fig. 4, the thin lines indicate the template and the thick  
10 lines indicate GsMTx-4.

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## CLAIMS

1. (Amended) A polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2, and forming an intermolecular disulfide bond between two of the cysteines contained in SEQ ID NO:1 or SEQ ID NO:2.
2. (Amended) A polypeptide or salts thereof comprising an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2 not consisting of an amino acid sequence represented by SEQ ID NO:4, and forming an intermolecular disulfide bond between two of the cysteines contained in the amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2.
3. (Amended) A polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2 of which one or several of the amino acids thereof have been deleted, substituted, inserted or added, forming an intermolecular disulfide bond and moreover showing mechano-sensitive channel inhibiting activity.
4. (Amended) A polypeptide or salts thereof described in the above claim 3 as consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2 of which one or several of the amino acids thereof have been deleted, substituted, inserted or added, of which the said amino acid sequence is an amino acid sequence represented by SEQ ID NO:16 or SEQ ID NO:17.
5. (Amended) A polypeptide or salts thereof consisting of an amino acid sequence represented by SEQ ID NO:16 or SEQ ID NO:17.
6. (Amended) A polynucleotide comprising the polynucleotide that encodes the polypeptide described in claim 1 as consisting of an amino acid sequence represented by SEQ ID NO:1 or SEQ ID NO:2 and forming an intermolecular disulfide bond between two of the cysteines contained in SEQ ID NO:1 or SEQ ID NO:2, of which the said polypeptide comprises the amino acid sequence represented by SEQ ID NO:2, encodes the polypeptide described in claim 3, or encodes the polypeptide described in claim 5.
7. A recombinant vector comprising the polynucleotide described in claim 6.
8. A transformant transformed with the recombinant vector described in claim 7.
9. A mechano-sensitive channel inhibitor comprising one or more of the polypeptides or salts thereof described in one of the claims from claim 1 to claim 5.
10. A remedy of atrial fibrillation comprising one or more of the polypeptides or salts thereof described in one of the claims from claim 1 to claim 5.